

MICROFLUIDIC DEVICES FOR CONTROLLED VISCOUS SHEARING AND FORMATION OF AMPHIPHILIC VESICLES

CROSS-REFERENCE TO RELATED APPLICATIONS

This application claims priority to U.S. Provisional Patent Application Serial No. 60/446,798, filed on February 11, 2003, entitled "Microfluidic Devices for Controlled Viscous Shearing and Formation of Amphiphilic Vesicles." The priority of this prior application is expressly claimed and its disclosure is hereby incorporated by reference in its entirety.

FIELD OF INVENTION

[0001] This invention relates to microfluidic devices and, more particularly, to microfluidic devices for controlled viscous shearing of oil-water emulsions of micro- and nano-scale droplets and the subsequent formation of amphiphilic vesicles such as liposomes, polymer vesicles, micelles, and the like. The invention further relates to systems and methods for post-assembly and post-processing of the droplets including droplet splitting, fusing and sorting.

BACKGROUND OF THE INVENTION

[0002] Current methods used to produce micro sized droplets include the generation of droplets from a capillary tip of a coflowing stream, a stream breakup under different shear flows, an extrusion through a polycarbonate membrane, and a breakup via induced surface instability of liquid jets. However, these methods do not allow for the simultaneous control of both the droplet generation speed and size. For size control, the only feasible method available has been to extrude emulsified droplets through microporous membranes or microchannels. The size of the droplets, however, are limited to the diameter of the channels or pores. In addition, the bulk

mixing processes tend not to allow for individual control of the composition of the droplet to generate precise multilamellar vesicles or asymmetric vesicles on demand.

[0003] Recent attempts have been made to generate droplets in microfluidic devices. These droplet generation devices are based on shearing mechanisms using the T-junction designs, flow focusing designs, interfacial tension driven method using microcapillary arrays, and the utilization of thin film electrode to meter droplets using either DEP, EWOD, or electrostatic forces. Most of these efforts focused on controlling droplet sizes but not the droplet generation rate. These devices have very little control over the pressure gradient for droplet formation and variations in the channels of these devices tend to cause drastic effects in the droplet formation process. As a result, none of these processes enable the fabrication of precise bi-layer vesicles in microfluidic devices.

[0004] Applications have emerged that can take advantage of precision generation of tiny droplets. These applications range from programmable biochemical assays to drug delivery particles. The control of droplet size and a tight droplet size distribution are paramount to these applications, such that the sizes of the droplets would determine the amount of drug encapsulation, polymer concentration and the drug release rate of the vesicle. The ability to control droplet generation speed would determine the analysis and production efficiency of the system. In addition, controlled assembly of multi-lamellar vesicles can encapsulate multiple reagents or drugs for broad applications in medicine and biology.

[0005] Thus, it would be desirable to provide systems and methods that facilitate greater control in the droplet formation process and, more particularly facilitate sheath flow to control viscous shear and pressure gradient during the process.

SUMMARY OF THE INVENTION

[0006] The present invention facilitates programmable control of size and composition of emulsified droplets, controlled multi-lamellar and asymmetric vesicles, 100% encapsulation of reagents (e.g. to save precious drugs and reagents), controlled insertion of membrane proteins, and sorting of vesicles/droplets. More particularly, the present invention is directed to microfluidic devices for controlled viscous shearing of oil-water emulsions of micro- and nano-scale droplets and the subsequent formation of amphiphilic vesicles such as liposomes, polymer vesicles, micelles, and the like. The present invention includes various microfluidic device designs for different nanomanufacturing features such as droplet formation speed, droplet size ranges, multi-lamellar amphiphilic layers, asymmetric vesicles and the like. Also included are designs for post-assembly and post-processing of the droplets including splitting, fusing, sorting and the like, and polymer emulsions. The combined features and functions of these devices enable the integrated amphiphilic vesicle production-line, all on a single microfluidic chip. These amphiphilic vesicles are artificial cells with applications in drug delivery (including biomolecular nanomedicine such as DNA, peptides, proteins), combinatorial chemistry, nanoscale chemical reaction chambers, biomolecular devices (power, optical, electrical), and various biosensors.

[0007] In one aspect, the present invention includes various microfluidic device embodiments for oil-water co-flows with tunable viscous shear forces higher than the immiscible interfacial tension forces to generate favorable conditions for droplet formation. The higher the shear force the smaller the droplet size. In the preferred embodiments, the design of the immiscible fluid sheath flow to establish stable interfaces with controllable shear forces advantageously enables

reproducible and batch fabrication of the devices, and the pressure gradient design advantageously generates favorable conditions for high speed droplet formation.

[0008] In another aspect, the present invention includes a method to control the size and generation rate of droplets/vesicles by controlling the flow rates of the immiscible fluid solutions.

[0009] In yet another aspect, the present invention includes a method to generate multi-layer membranes for artificial cells in microfluidic devices.

[0010] In a further aspect, the present invention includes various microfluidic post-assembly and post-processing methods to split droplets into smaller droplets, fuse droplets into larger ones, or sort the droplets based on density and size.

[0011] Other objects and features of the present invention will become apparent from consideration of the following description taken in conjunction with the accompanying drawings.

DESCRIPTION OF THE DRAWINGS

[0012] Figure 1 is a schematic layout of an amphiphilic vesicle “nanomanufacturing” production line with major components for directed self-assembly of droplets/vesicles and post-assembly manipulation such as sorting, splitting, fusing and the like.

[0013] Figure 2 is a schematic of a microfluidic device for directed self-assembly and nanomanufacturing of amphiphilic vesicles.

[0014] Figures 3A, 3B and 3C are photographs depicting the formation of a vesicle or droplet.

[0015] Figure 4A is a schematic of a cross-junction vesicle generator.

[0016] Figure 4B is a schematic of a fork-junction vesicle generator.

[0017] Figure 4C is a schematic of a bowtie vesicle generator.

[0018] Figures 5A-5C comprise photographs demonstrating the aqueous droplet formation in olive oil taken by a high speed camera in a device with a cross-junction vesicle generator design.

[0019] Figure 6 is a graph summarizing the results of this demonstration.

[0020] Figures 7A-7C comprise photographs demonstrating water-in-oil droplet formation in a microfluidic device with a bowtie vesicle generator design.

[0021] Figure 8 is a graph showing droplet generation rate for a given droplet size.

[0022] Figure 9 is a schematic of a bowtie vesicle generator coupled to a ladder channel.

[0023] Figure 10 is a schematic of a cross-junction vesicle generator with an expanded junction area and expanding outlet channel.

[0024] Figures 11A, 11B and 11C are photographs of the emulsion generated in the vesicle generator shown in Figure 10.

[0025] Figure 12 is a graph of droplet radius versus oil solution flow rate.

[0026] Figure 13 is a schematic of a microfluidic device for directed self-assembly and nanomanufacturing of multi-layer amphiphilic vesicles.

[0027] Figure 14 is a schematic of a multilayer amphiphilic vesicle.

[0028] Figure 15 is a schematic of a microfluidic device for multilayer droplet formation.

[0029] Figures 16A, 16B, and 16C are photographs of multilayer droplet formation using patterned hydrophobic/hydrophilic surfaces.

[0030] Figure 17 is a photograph of the formation of satellite droplets.

[0031] Figure 18 is a photograph of the generation of nanoparticles by a bifurcation junction.

[0032] Figure 19 is a collection of photographs showing a method of fusing droplets.

[0033] Figures 20A and 21B are schematics demonstrating a method of sorting.

[0034] Figures 21A, 22B and 22C are photographs showing a method of sorting satellite droplets.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENT

[0035] The present invention includes microsystem platforms that enable the automation of complex self-assembly processes. Turning to the figures in detail, Figure 1 shows a microsystem 10 that is embodied on a single microchip for droplet or vesicle formation 20 in which the droplet size and droplet formation speed can be controlled and which also includes designs for post-assembly and post-processing of the droplets (30, 40 and 50), which is discussed in greater detail below. Advantageously, the microsystem 10 of the present invention, and particularly the droplet or vesicle formation system 20, facilitates programmable control of size and composition of emulsified droplets, controlled multi-lamellar and asymmetric vesicles, 100% encapsulation of reagents (e.g. to save precious drugs and reagents), controlled insertion of membrane proteins, and sorting of vesicles/droplets. This microfluidic platform enables the control of concentrations

of solvents, lipids, co-block polymers and the like to alter size, shape, ionic strengths and composition of vesicle membranes to create artificial cells with desired functional properties. Droplets formed using the techniques of the present invention can be used as transporting and reaction vehicles for nanoliter to femtoliter volumes of reagents.

[0036] Turning to Figure 1, a preferred process for the formation of mono- and bi-layer amphiphilic vesicles 110 and 112 is shown. First, a sheath flow 125 of two types of solutions is formed at the junction 115 of three (3) inlet microchannels 122, 123 and 124 of a microfluidic device 120. As depicted, the sheath flow can be an immiscible fluid sheath flow such as an oil-water-oil flow or a water-oil-water flow. The sheath flow can comprise a three layered sandwiched flow. The relative flow rates of the oil and water through the microchannels 122, 123 and 124 are preferably controlled to dictate the viscous forces at the immiscible fluid or oil-water interfaces 127 and 129, which determine droplet formation rate and size. Second, by dissolving amphiphiles 150 in the oil solutions 155 a monolayer membrane 109 is formed around or encapsulates the droplets 108 to form monolayer vesicles 110. The monolayer vesicle 110 can then be delivered across another oil-water interface to form a bilayer vesicle 112 upon entering the aqueous solution 114.

[0037] As depicted, the central inlet microchannel 123 is an input microchannel for an aqueous solution 145 preferably comprising reagents 140. The reagents 140 can be of any form known in the art. In one embodiment, the reagents 140 include drugs or medicants which have been dissolved in an aqueous solution 145. Preferably the aqueous solution 145 comprises purified DI water.

[0038] The other two or outer inlet microchannels 122 and 124 are input microchannels for an oil solution 155 preferably comprising amphiphilic molecules 150. The amphiphilic molecules

150 can be lipids which have been dissolved in organic solvents. The amphiphilic molecules 150 can include olive oil, simple phospholipids such as oleic acid, complex phospholipids such as dipalmitoylphosphatidyl choline (DPPC) or dimyristoylphosphatidylcholine (DMPC), and the like. In a further embodiment, the amphiphilic molecules 150 can include surfactants such as SDS, Span, and Tween type surfactants that can stabilize interfaces. In yet another embodiment, the amphiphilic molecules 150 can include polymers. The polymers may include biodegradable polymers such as poly-lactic acid (PLA), poly-glycolic acid (PLG) or poly lactic/glycolic acid (PLGA). Polymers may also include UV curable polymers or thermally curable polymers. One skilled in the art would understand that the present invention is not limited to these examples. Further, the advantages of using one type of amphiphilic molecule 150 over another depends on the reagent 140 and desired application. In addition, as discussed further below, the two oil solution microchannels 122 and 124 may comprise more than one type of amphiphilic molecule 150.

[0039] Two general types of encapsulating methods are used to encapsulate the vesicle 108 with its monolayer membrane 109. The first method comprises a coating resulting from the combination of shearing forces and the solubility difference of lipids in the oil and in the water phase. For example, DPPC, DOPC and other lipids normally not soluble in oleic acid are dissolved into oleic acid by heating to create a clear uniform solution even at room temperature. As lipid interacts with water, the lipids spontaneously coats around the droplet created by shearing of the water and organic phase. In a similar fashion, copolymers may be dissolved in the organic solution. As the copolymers interact with the aqueous solution, the copolymers would be joined to coat the droplet created by the shearing of the water and organic phase.

[0040] The second encapsulating method comprises coating due to a chemical reaction that is caused by the reagents between the aqueous phase and the oil phase. Infusing the aqueous phase with a basic solution, such as CaOH_2 , NaOH or other basic reagents known to those of skill in the art, and the organic or oil phase with reactants such as alginate or oleic acid generates encapsulation emulsions with a shell surface comprised of the products from the chemical reaction.

[0041] Either of these two processes produces monolayer vesicles 110 which can be further processed to create bi-layer vesicles 112. The monolayer coated emulsion provides sufficient stability to be processed later into a variety of bilayer emulsions including liposome and asymmetric vesicles, as discussed in detail in regard to Figures 13 and 14.

[0042] The formation of a monolayer vesicle 110 generally occurs adjacent the junction 115 of the microchannels 122, 123 and 124 in the downstream output channel 128. Preferred embodiments of the present invention are designed to nanomanufacture droplets by controlling the interfacial tension and shear gradients in between oil and water. The monolayer droplet formation technique of the present invention involves the control of both the magnitude and type of flow through the three microchannels 122, 123 and 124 to control droplet size and uses the differences in the flow rates between the water and oil phases to control the speed of droplet or vesicle generation.

[0043] Specifically, the droplet breakup mechanism known as end-pinching occurs when high shear forces being applied to a droplet suddenly stop. Due to relaxation of the flow, i.e., as the co-flow of layers of oil and water enters an expanded channel junction 115 or output channel 128, the internal pressure gradient inside the droplet induces surface instability and breaks off the droplet in the process. In the channel junction 115, the flow before the output channel 128 acts

as the initiator for producing the high shear on the droplet 108. As the flow passes through to the output channel 128, the flow immediately decelerates producing the droplet end-pinching effect. Depending on the pressure gradients desired, the geometries of the three microfluidic channels 122, 123 and 124 and the output channels 128 may be altered.

[0044] Figures 3A, 3B and 3C, which are a sequence of photographs showing the formation of a droplet/vesicle in a cross-junction vesicle generator, provide a detailed depiction of the droplet breakup mechanism. The droplet generation process can be roughly divided into 3 stages: contraction (Figure 3A), relaxation (Figure 3B), and separation (Figure 3C). During the contraction phase, as the water (W) and oil (O) stream flow through the channel junction 115 and into the output channel 128, the curvature of the water tip increases to equilibrate with the pressure of the oil phase. During the relaxation phase, the change in curvature soon forms a necking region 111 that creates a pressure gradient at the tip of the stream. With the combination of the shear force from the oil stream, the net pressure inside the droplet 108 and the changes in the curvature of the surface, the droplet 108 detaches from the stream as shown in the separation phase (Figure 3C).

[0045] Using this droplet breakup mechanism, droplets have been formed which range in size from 150 μm to less than 1 μm in diameter. Droplet generation rates using these techniques range from 100 msec/droplet to less than 0.2 msec/droplet.

[0046] Simultaneous control of droplet size and generation rate may be achieved using the devices and methods of the present invention. Normally, the larger the droplet the slower the generation rate which may be a limitation to the throughput of the device. However, since droplet size is controlled by the flow rates of the outer inlet channels to generate shear forces and the central inlet channel can control the filling up of the droplets (or generation rate), by

increasing the flow rate of all the channels, or additionally, by increasing the channel size, the generation rate for larger droplets can be optimized. It is possible to map out the droplet size versus generation rate plot to identify the space to control both simultaneously. Figure 8 is such a plot for one particular device that can be used to determine the droplet generation rate for a give droplet size. D/D_i is the ratio of the diameter of the droplet over the width of the channel ($40\ \mu\text{m}$) and time is defined per droplet.

[0047] The relative flow rates of the three microchannels 122, 123 and 124 are preferably controlled to dictate the viscous forces at the oil-water interfaces, which determines droplet formation and size. In addition, the size of the droplets formed in the present invention can be controlled by varying the viscosity of the aqueous and oil solutions, the flow rates of these solutions, and the size of the channels. When the droplet sizes are equal or smaller than the width of the output microchannel 128, droplet size is consistent with the capillary number (Ca), which is described as the balance between interfacial tension and shear force. The actual capillary number varies with the flow types applied to the droplets.

$$Ca = \frac{\eta_c \gamma a}{\sigma} \quad (1)$$

[0048] In the expression above, η_c is the viscosity of the continuous phase, γ is the shear rate of the continuous phase, and the product of the two is equal to the magnitude of the shear force that drives droplet breakup. Opposing the shear force, the capillary force acts to keep the droplet from deformation and is equivalent to the ratio of a , the radius of the droplet divided by σ , the interfacial tension between water and oil interface.

[0049] The shear rate of oil in the channel can be estimated as:

$$\gamma = \frac{Q_o}{AW_c} \quad (2)$$

[0050] A , W_c , and Q_o are the cross sectional area of the channel, the width of the channel, and the flow rate of the oil phase. Incorporating eq(1) into eq(2) yields a formulation relating the oil flow rate to the generated droplet radius.

$$a = \frac{W_c A \sigma}{\eta_c Q_o} \quad (3)$$

[0051] As depicted in Figure 12, Equation (3) indicates that the radius of the droplet varies inversely with the oil flow rate. Expanded junction designs, such as the diamond shape shown in Figures 3A-C and 10, or those inherent in the bowtie designs shown in Figures 4C, 7A-C and 9, enable higher oil flow rates.

[0052] The control of the rate of droplet generation is achieved through adjusting the infusion rate of the water phase. The droplet generation rate reflects the different time scales in the 3 stages of droplet formation process illustrated earlier. Prior to the contraction phase, the time required for the water stream to fill a droplet with size that equilibrates with the surrounding oil pressure is characterized by T_f . During the contraction phase, the viscous stress shears the surface of the water stream and the time of shearing is T_γ . Lastly, after the contraction phase, as the droplet reaches the widened channel, the shear rate of the oil phase is weakened and the restoration time of the capillary force, T_σ , is required to pinch off the droplet. The three time scales are expressed below as:

$$T_f = \frac{4\pi a^3}{3Q_w} \quad (5)$$

$$T_\gamma = \gamma^{-1} \quad (6)$$

$$T_\sigma = \frac{\eta_c a}{\sigma} \quad (7)$$

[0053] When $Q_w=Q_o$ (Q_w , water phase flow rate; Q_o , oil phase flow rate), the maximum difference in droplet size is approximately 10% allowing the different time factors of droplet generation to be compared. According to Table I, the value of T_f exceeds all other time scales, and as such, T_f dominates the actual formation time. From equation (5) this scaling would predict that the droplet generation rate varies inversely to the flow rate of the water phase.

[0054] Table I: Time of breakup, time of formation and the experimental time of droplet generation are shown.

$Q_w = Q_o$ ($\mu\text{l}/\text{min}$)	T_f (ms)	T_r (ms)	T_σ (ms)	T_{exp} (ms)
1	30.154	3.855	0.087	15.5 ± 0.5
0.8	37.692	4.819	0.087	20 ± 2
0.6	59.232	6.426	0.092	26 ± 2
0.4	66.212	9.639	0.084	44 ± 2
0.2	144.490	19.277	0.862	128 ± 8

[0055] The flow rate for the oil microchannels 122 and 124 and for the aqueous microchannel 123 are preferably controlled by a pump. The pump can be a syringe pump, an on-chip syringe pump, a pressure-driven pump, or any other pump known in the art. In a further embodiment the flow rates may be controlled by a magnetohydrodynamic (MHD) fluidic manifold, that can individually control the pressure/flow rates of each microchannel. Typically, flow rates are in the range of about $-0.5 \mu\text{l}/\text{min}$ to $-20 \mu\text{l}/\text{min}$, however, the present invention is not limited to these ranges.

[0056] As depicted in Figure 2, the microfluidic device 120 preferably comprises at least three inlet microchannels 122, 123 and 124 used to direct co-directional flows of oil and aqueous solutions toward a channel junction 115 and into an output channel 128. In an alternative embodiment, the junction 115 can comprise more than three inlet microchannels.

[0057] The microchannels 122, 123, and 124 of the microfluidic device 120 are preferably molded out of PDMS in an SU-8 mold or are injection molded from a variety of moldable plastics known in the art such as polystyrene or polycarbonate. Other microfabrication materials such as silicon or glass can also be used since photolithography and etching can define microchannels on those substrates. The width of the microchannels 122, 123 and 124 are generally in the range of about 20 μm to 100 μm and more preferably in the range of about 30 μm to 50 μm . As the widths of the microchannels drop below 20 μm towards 1 μm and below, the microchannels are preferably formed using micro-lithography techniques.

[0058] Once the microchannels 122, 123 and 124 are formed, the microchannels are then bonded to a substrate to form a microfluidic chip. The substrate is preferably formed from glass, polystyrene, PDMS or the like. Though generally in the range of about 1 mm to 7 mm, the size of the microfluidic chip can vary depending on the application.

[0059] As depicted in Figures 4A, 4B and 4C, the configuration of the inlet microchannels, the channel junction and the outlet channel can vary. Some exemplary embodiments for generating droplets or amphiphilic vesicles include (a) a cross-junction vesicle generator 220 shown in Figure 4A, (b) a fork-junction vesicle generator 320 shown in Figure 4B, and (c) a bowtie vesicle generator 420 shown in Figure 4C, each representing a different condition and control on the interfacial tension and the shear rates.

[0060] The angle of entry θ_A and θ_B for the two inlet oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the inlet aqueous solution microchannel (123, 223 and 323) is generally in the range from about 0 to about 90 degrees (Figures 2, 3A-C, and 4A-B).

Preferably, the angle of entry θ_A and θ_B for the two inlet oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the inlet aqueous solution microchannel (123, 223 and

323) is between about 10 and 60 degrees and most preferably is in the range of approximately 25 to 40 degrees. The angle of entry θ_A and θ_B for both oil flow microchannels (122 and 124, 222 and 224, 322 and 324) relative to the aqueous solution microchannel (123, 223 and 323) can be equal. Alternatively, the angle of entry θ_A and θ_B for one of the two oil flow microchannels (122 and 124, 222 and 224, 322 and 324) can be greater than the angle of entry θ_A and θ_B for the other oil flow microchannel (122 and 124, 222 and 224, 322 and 324).

[0061] Figures 5A-5C are photographs demonstrating the water-in-oil aqueous droplet formation method in the cross-junction vesicle generator 220 illustrated in Figure 4A. These photographs demonstrate the end-pinching droplet formation which occurs because of the applied shear forces. The microchannel width of the T junction or cross-junction microfluidic device used in the experiment is 40 μm . In Figure 5A, the aqueous solution flows from the horizontal channel 223 from right to left. Shear forces are applied from each of the two vertical channels 222 and 224. In Figure 5B, a droplet 208 has pinched off the tip of the aqueous solution. In Figure 5C, the droplet travels across the horizontal output channel 228. Displacement of droplets released from the T junction is shown plotted as the function of time in Figure 6. Initially the 'seed' drop nucleates for a small time, then the drop slowly grows and finally, after the release, the drops moves faster. This corresponds to the plateau region in the curve, small-sloped line and large-sloped line. The inset curve shows the number of droplets released as a function of time. The straight line implies the droplets are released at equal intervals of time.

[0062] Figures 7A-7C comprise photographs demonstrating water-in-oil droplet formation in the bowtie microfluidic device 420 illustrated in Figure 4C. In Figure 7A, a water stream 445 is sandwiched by oil flow 455 as the stream forms a necked region 411 adjacent the necking junction 426 and expands to start forming a droplet 408 in the output channel 428. As the ratio

between oil and water flow rate is increased, the interfacial tension can no longer sustain a laminar flow line. As a result, droplets 408 with a diameter of approximately 25 μm are formed (Figure 7B). Droplets with a diameter of approximately 150 μm are shown extruding through the necking junction 426 at about 90 droplets per second in Figure. 7C.

[0063] The “cross-junction device” (FIGS. 6A-6C) generates droplets at 1-2 drops/sec whereas the bowtie device (FIGS. 7A-7C) was much faster at 90 drops/sec. The cross-junction droplet generation rate can be increased in the cross-junction generator with higher flow rates.

[0064] Figure 9 illustrates a vesicle generator incorporating an expanded junction. More particularly, the emulsion generation device 500 includes a bowtie vesicle generator 420 coupled to a ladder microchannel 520. The device 500 is designed to control the local shear forces between the co-flow of organic and aqueous boundary interface to generate monodispersed droplet emulsions.

[0065] Figure 10 provides another exemplary embodiment of a cross-junction vesicle generator 220' having inlet channels 222, 223 and 224 and an outlet channel 228, and incorporating an expanded junction 215 design. As shown in Figures 11A and 11B, emulsions were generated at the cross junction 215 allowing the size of the droplet emulsion to be manipulated by the flow rate of the oil phase. As indicated in Figure 12, the radius of the droplet varies inversely with the flow rate of the oil phase. Under this design emulsions as small as 0.5 μm in radius have been generated as shown in Figure 11C.

[0066] As depicted in Figure 13, after formation, the monolayer vesicle 110 is delivered across an oil-water interface 130 to form a bilayer vesicle 112 upon entering the aqueous solution 114. The oil-water interface 130 is formed by water or an aqueous solution flowing through two water inlet channels 132 and 134 and an outlet channel 138. The amphiphilic molecules 135 of the oil-

water interface 130 may be the same type of amphiphilic molecules 150 as in the oil microchannel solvent 155. It is also possible to generate asymmetry in the bi-layers such that the inner and outer amphiphilic molecules are different. This is significant since biological membranes are, in fact, a fluid composition of lipids and other amphiphilic molecules. It will be possible to dynamically load the bi-layer amphiphilic vesicles 112 with different molecules, both in the membrane and encapsulated within it. Asymmetric vesicles are created through post encapsulating monolayer coated vesicles 110 with a second or more layers of encapsulating agents including alginate, lipids, polymer, surfactants and the like. For example, an oleic acid coated monolayer vesicle 110 may be encapsulated by alginate gels 113 to form a hard shell around the permeable oleic acid monolayer 109. These vesicles will allow multiple drugs to be encapsulated inside a single unit providing a platform for a multiple drug delivery system.

[0067] Turning to Figure 15, multi-layer droplets side extrusion channels may be formed. In this embodiment, a large droplet 216 is generated in a generation channel 214. The large droplet 216 is later sheared by another flow channel 218 perpendicular to the generation channel 214. As a result of this shearing, a second liquid encapsulates the large droplet 216 to form a two layer droplet 212. This process may be repeated as desired to generate multi-layer droplets in a continuous fashion.

[0068] Multi-layer droplets may also be formed using patterned hydrophobic/hydrophilic surfaces. Multi-layered droplets, or compound drops, are created in microfluidic devices through controlling the surface properties of the microfluidic channels. Microchannels with hydrophilic surfaces allows water to spread and resist the spreading of oil. In contrast, microchannels with hydrophobic surfaces allow oil to spread and repels the spreading of water. Using a combination of these two surfaces, a compound, multi-layer droplet is generated through sequential

generation of one type of emulsion followed by a second generation in an opposite medium. For example, a compound drop of water-oil-water is generated from water in oil emulsion followed by the generation of oil in water emulsion (Figure 16A). If polymers, lipids, or other chemical compounds are dissolved or dispersed in the medium, once it is encapsulated inside a compound droplet this will produce multilayered vesicles because of the stability of the encapsulated compounds. Alternatively, since surfactant can act as a stabilizing agent between immiscible surfaces, a compound droplet can also be created through using a combination of water soluble and oil soluble surfactants as shown in Figures 16B and 16C.

[0069] Returning to Figure 1, which illustrates the droplet post-assembly and post-processing components in one integrated design, is a schematic layout of an amphiphilic vesicle “nanomanufacturing” production line 10 with major components for directed self-assembly of droplets/vesicles 20 and post-assembly manipulation such as sorting 30, splitting 40, fusing 50 and the like. Manipulation of the droplets may be advantageous to prepare the droplets for digital bioassays, screening, or for drug delivery.

[0070] In particular for droplet generation 20, droplets 26 are shown formed in a cross-junction device having oil and water solution inlet channels 22, 24 and 23, and an output channel 28. For splitting 30, droplets 26 enter a bifurcation 35 of the microfluidic channels and are pressurized into both microchannels 32 and 34 at this junction 35. The inlet channel 28 is connected to two or more daughter channels 32 and 34. The width of the daughter channels may vary depending on the desired application, but the width is generally in the range of 1 μm to 100 μm . A pressure gradient can be created by differences in the pressures in the inlet channel 28 and daughter channels 32 and 34. The pressures can be controlled by altering the width and/or length of the parent and daughter channels 28, 32 and 34 or by modifying the external pressure, for example

by applying higher flow rates. The two pressures stretch the droplets 26 and the corner 37 of the channel walls assists in shearing apart the droplets 26. Variations in the size of the daughter microchannels 32 and 34 can also be used to create split droplets of equal or unequal size. For example, in a splitter system with two daughter channels 32 and 34, one daughter channel 32 may have a width of approximately 40 μm and the other daughter channel 34 may have a width of approximately 60 μm . This splitter system 30 allows for the creation of two droplets which are of different sizes. Nanoparticles may also be generated by a bifurcation junction with unequal daughter channels. Through unequal fission, droplets with very small diameters may be created as shown in Figure 18. Droplets as small as less than 1 μm have been generated from the splitting of 30 μm droplets.

[0071] For the sorter 40, droplets passing from left to right are sorted by velocity. Again, at the bifurcation 45, there are two channels 44 and 46 with different pressure drops to “attract” the droplets. The higher the velocity, the more likely the droplet is able to cross-over to the horizontal channel. Since the smaller droplets 49 have higher velocity they will be collected in the horizontal channel 44 and the larger ones 48 will drop into the vertical channel 46.

[0072] Droplets of different sizes may also be sorted as shown in Figures 21A and 21B. In this embodiment, droplets may be sorted by having an input channel 262 meet two daughter channels 264 and 266 at a T-junction. The streams 268 which direct a droplet to the lower daughter channel 264 or upper daughter channel 266 may be modified based on the relative flow rates of the two daughter channels 264 and 266. In Figure 20A, the complete suspension of the droplet 267 in the lower stream moves the droplet 267 to the bottom channel 264. If the flow rate of lower channel 264 is increased, the number of streams 268 moving toward the lower channel

increases (Figure 20B). This increases the droplet size that can be removed by the velocity gradient between the upper daughter channel 266 and the lower daughter channel 264.

[0073] Satellite droplets 281 can be separated from larger droplets 284 as shown in Figures 21A-22C. Satellite droplets 281 of less than 1 μm in diameter can be generated, separated and then collected in the microchannel without using surfactants. Large and small satellite droplets are first generated in the microchannel (Figure 21A). The arrow indicates the direction of flow and the dash circles indicate the presence of a satellite droplet 281. Satellite droplets 281 are carried by the flow into the upper channel 288, while large droplets 284 are pulled by shear and pressure forces into the lower channel 285 (Figure 21B). Only satellite droplets 281 are observed in the collecting zone 289 for the upper channels 288 (Figure 21C). The flow rates used for this example were 1.7 $\mu\text{L}/\text{min}$ for water and 3.65 $\mu\text{L}/\text{min}$ for oil.

[0074] In order to fuse two or more droplets together, two or more input channels 44 and 54 may be used which intersect. The intersection 55 of the two or more input channels leads to an output channel 52 for the fused droplet. For example, in a system with two input channels larger droplets enter a bifurcation joint 55 from the right hand side and get trapped due to smaller size of the neck of the vertical channel 52. Smaller droplets enter from the left hand side and are able to enter the vertical channel 52. In the process, the larger droplets experience an oscillating or pulsatile pressure that causes the droplets to rock back and forth. This rocking motion “hammers” two droplets together to form the fused droplet. The fused droplet then travels down the vertical channel 52.

[0075] Droplets may also be fused based on a velocity gradient profile as shown in Figure 19. Droplet fusion occurs when the fluids between the droplets are drained. This can occur either by an induced velocity gradient between the droplets or by removing fluids in between droplets at a

bifurcating junction. Using the droplet focusing design, the droplets are focused into the lateral channels. The droplets are focused into the middle channel 254 due to the cancellation of the force moving in the lateral direction. The net force exerted by the inlet flow moves the fused droplet 256 into the desired middle outlet channel 254. Through controlling the width of the junction gap and the separating flow rates of the lateral channels, droplets can be fused at desired numbers and rates.

[0076] Turning to Figure 17, nanoparticles may be generated by satellite droplet generation. Satellite droplets 230 are created by the residues of the primary droplet 240 after breakup. Satellite droplets are generally created when higher water flow rates are used for the droplet generation system such as that described above. When satellite droplets are generated, the size of the satellite droplets 230 are controlled by varying the generation conditions for the primary droplets 240 such as by altering the flow rates of the oil and/or water phase. These satellite droplets 230 generally have a volume that is less than one percent of the volume of the original droplet 240. Satellite droplets have been generated both from water in oil emulsion systems and oil in water emulsion systems. A typical water in oil satellite droplet is shown in Figure 17.

[0077] As indicated, complex layered vesicles may be created through a microfluidic system in accordance with the present invention that is capable of controlling and rectifying the size of emulsions. The system of the present invention may create a user defined liposome with controllable parameters including colloidal size, shape, surface properties, membrane properties, high encapsulation efficiency, programmed cell to cell recognition properties and biocompatibilities. In the medical field, applications include gene and protein therapy, cancer chemotherapy, and other clinical treatments that involve targeted drug delivery mechanisms. In computation science, individual liposomes may be designed to have specific surface and integral

properties to form a unit that express live computational algorithms. In electrical sciences, cell batteries, conductors, and switches may be created from liposome with specific membrane permeability and conductance that respond to signal cues.

[0078] Many arrangements of amphiphilic vesicles can be explored that mimic real biological cells and their networks. The system of the present invention has the potential to implement many of them. For example, a nanotube-vesicle network that mimics the Golgi complex in intracellular transport of protein and lipids was recently developed. Combinatorial synthesis using amphiphilic vesicles can be carried out by sorting and fusing amphiphilic vesicles with reagents and samples, providing a digital chemistry factory for synthesis and nanoassays. By inserting various tags and biomarkers, it is also possible to use precisely sized amphiphilic vesicles for particle imaging.

[0079] While the invention is susceptible to various modifications and alternative forms, a specific example thereof has been shown in the drawings and is herein described in detail. It should be understood, however, that the invention is not to be limited to the particular form disclosed, but to the contrary, the invention is to cover all modifications, equivalents, and alternatives falling within the spirit of the disclosure.